

Infection of Human Immunodeficiency Virus 1 Transgenic Mice with *Toxoplasma gondii* Stimulates Proviral Transcription in Macrophages In Vivo

By Ricardo T. Gazzinelli,^{*||} Alan Sher,^{*} Allen Cheever,^{*} Susan Gerstberger,[‡] Malcolm A. Martin,[§] and Peter Dickie[§]

From the ^{*}Immunobiology Section, Laboratory of Parasitic Diseases, [‡]Laboratory of Immunoregulation, and [§]Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and the ^{||}Department of Biochemistry and Immunology, Federal University of Minas Gerais, 31270-010 Belo Horizonte, Brazil

Summary

Human immunodeficiency virus (HIV) 1 transgenic mice expressing low or undetectable levels of viral mRNA in lymphoid tissue were infected with the intracellular protozoan *Toxoplasma gondii*. Exposure to this parasite resulted in an increase in HIV-1 transcripts in lymph nodes, spleens, and lungs during the acute phase of infection and in the central nervous system during the chronic stage of disease. In vivo and ex vivo experiments identified macrophages as a major source of the induced HIV-1 transcripts. In contrast, *T. gondii* infection failed to stimulate HIV-1 transcription in tissues of two HIV-1 transgenic mouse strains harboring a HIV-1 proviral DNA in which the nuclear factor (NF) κ B binding motifs from the viral long terminal repeats had been replaced with a duplicated Moloney murine leukemia virus core enhancer. A role for NF- κ B in the activation of the HIV-1 by *T. gondii* was also suggested by the simultaneous induction of NF- κ B binding activity and tumor necrosis factor α synthesis in transgenic mouse macrophages stimulated by exposure to parasite extracts. These results demonstrate the potential of an opportunistic pathogen to induce HIV-1 transcription in vivo and suggest a mechanism for the in vivo dissemination of HIV-1 by macrophages.

The progression towards AIDS in HIV-1-positive individuals appears to be directly related to HIV-1 replication and virus load in lymphoid tissues (1–3). In vitro studies have demonstrated that activation of host immune cells (i.e., CD4⁺ T cells and macrophages) facilitates multiple steps of the virus life cycle (i.e., entry, integration, RNA synthesis, assembly, and/or release) in both primary and chronically infected cells (3). Thus, stimulation of the immune system may have an important influence on viral replication and progression of disease in HIV-1⁺ individuals. HIV-1 induction is regulated at the transcriptional level by *cis*-elements in the 5' LTR of integrated HIV-1 proviral DNA (4, 5). Several mechanisms by which the HIV-1 LTR can be activated and virus replication promoted have been demonstrated in vitro, a primary pathway involving the action of cytokines on infected host cells (3, 6–12). Events that induce cytokine expression in vivo such as concomitant infections might, therefore, enhance HIV-1 transcription affecting the levels of cell-free and cell-associated virus and possibly accelerate the progression to AIDS (13–16).

Toxoplasma gondii, an opportunistic pathogen, is frequently associated with HIV-1 infection, and is a common cause of encephalitis in AIDS patients (17, 18). Previous

studies have indicated that infection with this protozoan parasite induces strong cell-mediated immune responses (19) and that live tachyzoites as well as tachyzoite extracts can directly trigger human/mouse monocytes or macrophages to produce a series of monokines (GM-CSF, TNF- α , IL-1, IL-6, and IL-12; 19–23) among which are known enhancers of HIV-1 expression in vitro (6–11). Indeed, recent experiments performed in our laboratories indicate that activation of human primary macrophages with *T. gondii* promotes the replication of a monotropic strain of HIV-1 in vitro (24).

In the present study, the ability of *T. gondii* to trigger HIV-1 transcription in vivo was investigated in recently developed transgenic mice incorporating the entire HIV-1 proviral genome (25). HIV-1 transgenic mice have been used in the past as models of HIV-1 disease (25–27) but their application in defining the in vivo regulation of HIV-1 expression in lymphoid cells by another infectious agent has not been reported. The results presented here show that infection with *T. gondii* enhances expression of HIV-1 RNAs in the spleens, lymph nodes, and lungs during acute infection, and in brain tissue during chronic disease. The enhanced expression of HIV-1 transcripts appears to be lo-

calized to macrophages and is associated with the induction of host monokines by the parasite. Our results demonstrate the potential of an opportunistic pathogen to promote HIV-1 expression and provide an experimental model for the development of therapies that prevent activation of HIV-1 5' LTR and therefore retrovirus transcription *in vivo*.

Materials and Methods

Derivation and Characterization of HIV-1 Transgenic Mice. Transgenic animal hosts were derived from FVB/N mouse (a murine inbred strain) embryos (28) after the pronuclear microinjection of HIV-1 proviral DNA. One transgenic mouse line (166) contained the full-length, wild-type HIV-1 DNA; two independent derivatives (Hm28 and Hm54) of a second line, designated Moloney murine leukemia virus/HIV-1[Δ nuclear factor- κ B] (MLV/HIV-1[Δ NF- κ B]),¹ harbored HIV-1 proviruses in which the NF- κ B binding motifs in the HIV-1 LTR were replaced with core enhancer sequences of MLV (see Fig. 1). This replacement was originally designed to augment the very low levels of expression directed by the wild-type HIV-1 LTR in mouse cells or in previously described transgenic mice. In the present study, transgenic animals containing the MLV/HIV-1[Δ NF- κ B] proviral DNA were used instead, as negative controls, to test the involvement of NF- κ B binding sites within the HIV-1 LTR on *T. gondii*-induced viral mRNA expression. These mice were generated using protocols described in detail elsewhere (29). Briefly, PCR-amplified MLV enhancer sequences were cloned into an XbaI site engineered by M13 mutagenesis at the position of a NF- κ B deletion in the wild-type HIV-1 LTR. The recombinant proviral DNA was then assembled by the multiple ligation of genomic DNA fragments.

The three transgenic lines stably transmitted their respective transgenes in Mendelian fashion (at 20–60 copies present at single integration sites) and expressed HIV-1 RNA in multiple tissues including secondary lymphoid organs (25). Each proviral DNA encoded the full complement of HIV-1 genes and directed the synthesis of three size classes of viral mRNAs corresponding to unspliced (*gag/pol*), singly spliced (*vif*, *vpr*, *env*) and multiply spliced (*tat*, *rev*, *nef*) HIV-1 transcripts (see Fig. 1). In contrast to the HIV-1 RNA patterns commonly associated with acutely infected human lymphocytes but reminiscent of a Rev-deficient phenotype (30), HIV-1 RNA in mouse tissue was often dominated by the presence of small molecular weight (2-kb class), multiply spliced transcripts. HIV-1 RNA sizes differed slightly between the wild-type HIV-1 transgenic line 166 and the MLV/HIV-1[Δ NF- κ B] recombinant lines (Hm28 and Hm54) because of the presence of MLV enhancer sequences in the LTR that elongated transcripts from the recombinant LTR by ~200 bases (see Fig. 1 B). Experiments were conducted in heterozygous and homozygous line 166 mice, whereas only heterozygous Hm28 and Hm54 mice were available for use since homozygous animals in the latter lines are nonviable.

¹Abbreviations used in this paper: H&E, hematoxylin and eosin; HPRT, hypoxanthine phosphoribosyltransferase; MLV, Moloney murine leukemia virus; MLV/HIV-1[Δ NF- κ B], recombinant HIV-1 containing a replacement of the two NF- κ B binding sites from viral LTRs for a duplicated enhancer from MLV core; NF- κ B, nuclear factor- κ B; SAG-2, *T. gondii* surface antigen 2; STAg, soluble toxoplasma antigen.

Experimental Infections with *T. gondii*. HIV-1 and MLV/HIV-1[Δ NF- κ B] transgenic mice as well as FVB/N nontransgenic control animals were infected at 6–8-wk-old by intraperitoneal inoculation with 20 cysts of ME-49 an avirulent strain of *T. gondii*. The cysts had been obtained from brains of donor mice infected at least 1 mo earlier as described previously (31).

Histopathology. At 8–10 (acute stage) or 50–70 d (chronic stage) postinfection animals were killed by cervical dislocation. The lungs and brains of each animal were separated into two lobes or halves, one of which was fixed in formalin for histopathology while the second was used for RNA extraction (see below). After 24 h in formalin, the tissues were transferred to 70% ethanol before processing for paraffin sections. The resulting slides were stained with hematoxylin and eosin (H&E) and the sections were evaluated microscopically as described previously (30).

Cell Separation Techniques. Pooled spleen cells collected from mice 10 d after infection with *T. gondii* were passed through a column for T cell enrichment (MTCC-100; R&D Systems, Inc., Minneapolis, MN) or adhered in tissue culture-treated plastic for isolation of macrophages (21, 22). FACS[®] analysis of spleen cells using anti-CD3 and anti-MAC1 mAbs (Becton Dickinson & Co., Immunocytometry Systems, San José, CA) were used to verify the enrichment of T cells or macrophages in the different splenocyte populations. Dead cells were excluded by propidium iodide staining.

To prepare inflammatory macrophages, peritoneal exudate cells were obtained 5 d after i.p. injection of 1.5 ml of 3% thioglycolate and plated at 2×10^6 cells/ml in 24-well flat-bottom plates (Costar Corp., Cambridge, MA). After 4 h of incubation (37°C, 5% CO₂), the nonadherent cells were removed and the macrophage-enriched monolayer was washed in warm medium (21, 22).

In Vitro Stimulation of Macrophage Monolayers. A soluble extract of *T. gondii* tachyzoites (soluble toxoplasma antigen [STAg]) was obtained by sonication and centrifugation of tissue culture-derived RH strain tachyzoites as previously described (31). To measure monokine responses and/or induction of HIV-1 proviral transcription, STAg (5 μ g/ml), *Escherichia coli* LPS (23; 100 ng/ml), or TNF- α (100 U/ml; Genzyme Corp., Cambridge, MA) were added in 1 ml of medium (DMEM, 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, 25 mM HEPES, and 50 μ M β -ME) to monolayer wells in the presence of either control (GL-113 anti- β -galactosidase; 20 μ g/ml) or anti-TNF- α (20 μ g/ml) mAbs as indicated. The supernatant and adherent cells were obtained 8–12 h after stimulation for measurement of TNF- α or detection of actin or HIV-1 mRNAs, respectively.

RNA Preparation. RNA was isolated from cells and tissues using an adaptation of a published procedure (32). Total splenocytes (10^7), fractionated spleen cell populations (2×10^6), thioglycolate-elicited macrophages (2×10^6 before adherence), or ~200 mg of tissue (spleen, lung, or brain) were homogenized in 1 ml of ice-cold acid guanidinium thiocyanate solution buffer supplemented with B-ME (0.7%). After addition of 0.1 ml of 2 M sodium acetate, pH 4.0, 1.0 ml of water-saturated phenol, and 0.2 ml of chloroform, the samples were mixed vigorously and left on ice for 15 min. RNA was recovered in the aqueous phase after centrifugation (10,000 rpm at 4°C) and precipitated in isopropanol as previously described (32). The final RNA pellet was resuspended and stored frozen in water containing either 1 mM EDTA for reverse transcriptase (RT)-PCR or 0.2% SDS for Northern blot analysis. In some experiments, poly(A) RNA was isolated from total RNA samples by oligo-dT cellulose chromatography.

Northern Blot Analysis of Proviral Transcripts. RNA samples (20 μ g/lane) were fractionated by electrophoresis through agarose (1.25%) under denaturing conditions (2.2 M formaldehyde), blot-

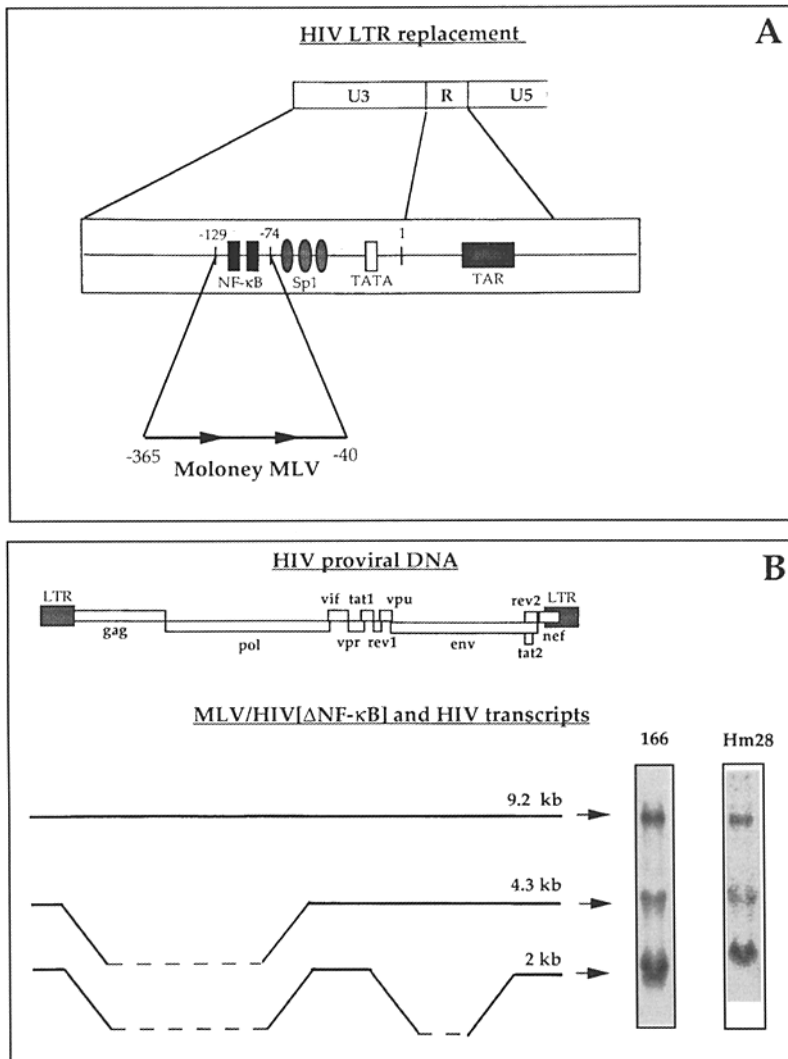


Figure 1. (A) Transgenic mice were created by pro-nuclear microinjection of FVB/N embryos with either the wild-type HIV-1 proviral DNA (line 166) or recombinant MLV/HIV-1[Δ NF- κ B] proviral DNA (lines Hm28 and Hm54) in which the two NF- κ B binding motifs in the U3 region of the HIV-1 LTR between nucleotides -129 and -74 (numbered relative to the transcription start site) were deleted and replaced with enhancer sequences from the MLV (Moloney MLV) according to a previously described cloning protocol. (B) Comparison by Northern analysis of HIV-1 RNAs extracted from eye lens (a site of high level constitutive expression) of line 166 and Hm28 mice. The three species of HIV-1 RNA correspond in approximate size to genomic and spliced HIV-1 transcripts typically observed in infected human cells. RNA transcripts from MLV/HIV-1[Δ NF- κ B] mice (line Hm28, splenocytes) are larger than those extracted from HIV-1 transgenic mice (line 166) because of the MLV insertion into the LTR region which extends the HIV-1 transcripts by \sim 200 bp.

ted onto nylon membranes, UV cross-linked, and hybridized to a 1.25-kbp HindIII Nef cDNA fragment (26, 27). Blots were washed under stringent conditions ($0.1 \times$ SSPE, 0.5% SDS, 65°C) and visualized by phosphoimage analysis (model BAS2000; Fuji Medical Systems, Stamford, CT). Membranes were stripped by boiling in $0.1 \times$ SSPE, 0.5% SDS and reprobbed with a human actin probe (Life Technologies, Baltimore, MD) for measurement of a housekeeping transcript. RNA molecular weight markers (Life Technologies) were used to identify 18S and 28S RNA species after ethidium bromide staining of the agarose gels.

Electrophoretic Mobility Shift Assay. Nuclear extracts of peritoneal macrophages from FVB/N or HIV-1 transgenic mice unstimulated or stimulated with either LPS (100 ng/ml), STAg (5 $\mu\text{g}/\text{ml}$), or TNF- α (100 U/ml) were prepared as previously described (33) and assayed for the presence of NF- κ B binding activity by electrophoretic mobility shift assay (EMSA) (34). Two double-stranded oligonucleotide probes were employed: a palindromic κ B probe (PD- κ B; 35) or an HIV-1-derived NF- κ B binding motif (HIV-1- κ B; 4). Approximately 0.4 μg of nuclear extract was used in each binding reaction. Antibodies directed against NF- κ B p65 protein, types C-20 or A (4 μl each; Santa Cruz Bio-

technology Inc., Santa Cruz, CA) were employed in the super-shift assays.

RT-PCR. 1 μg of total RNA obtained from either lung or brain of infected mice was reverse transcribed using MLV RT (GIBCO BRL, Gaithersburg, MD). The reaction mixture was then diluted 1:8 and 10 μl used for specific semi-quantitative amplification of cytokine mRNA using Taq DNA polymerase (Promega Corp., Madison, WI). Southern transfers of PCR products were subsequently probed with internal cytokine-specific oligonucleotides and visualized using the ECL chemiluminescent detection system (Amersham International, Amersham, Bucks, UK). Nucleotide sequences for sense and antisense primers and probes for hypoxanthine phosphoribosyltransferase (HPRT) 23, IL-1 27, IL-6 33, TNF- α 32, GM-CSF 35, and *T. gondii*-specific gene surface antigen (SAG-2) [40] have been previously described (21, 36) and the cycle numbers (indicated within the brackets) determined experimentally.

TNF- α Assay. TNF- α was quantitated in 8–12-h supernatants of adherent peritoneal macrophage cultures by means of a cytotoxicity assay using TNF-sensitive L929 cells and murine rTNF- α (Genzyme Corp.) as a standard (23).

Results

Increased HIV-1 mRNA Expression in Tissues of Transgenic Mice Infected with *T. gondii*. Infection with *T. gondii* initially manifests as an acute phase in which the tachyzoites (the rapidly multiplying stage of the parasite) are found in tissues accompanied by mononuclear inflammatory reactions leading to small necrotic foci. Parasite multiplication during this stage occurs preferentially in the liver, lungs, and lymphoid tissues (19, 37). Paralleling the tissue tropism of the parasite, expression of discrete HIV-1-specific RNAs was elevated in the lungs, spleens and lymph nodes (Fig. 2, top and Fig. 3, left) of transgenic mice (line 166) carrying a complete copy of HIV-1 proviral DNA and analyzed during that period (10 d after *T. gondii* infection). HIV-1-specific transcripts were identified on the basis of their size and comigration with HIV-1 RNA extracted from productively infected human cells (Fig. 3). By comparison, there was only a modest increase in HIV-1 transcripts in splenic extracts from a second infected transgenic mouse line (Hm28) in which the NF- κ B motifs in the LTR had been replaced with the core enhancer element from MLV

whereas no HIV-1 RNA was detected in lung tissue removed from the same animals (Fig. 2). Furthermore, identical results were obtained with a second independently derived transgenic line (Hm54) containing the MLV enhancer substitution (designated MLV/HIV-1[Δ NF- κ B]) (data not shown).

Histopathological changes were evident in the lungs of acutely infected animals regardless of strain (nontransgenic, HIV-1, or MLV/HIV-1[Δ NF- κ B] transgenic mice) and featured comparable levels of nodular infiltrates of lymphocytes and macrophages (generally $\sim 70 \mu\text{m}$ in diameter) as well as perivascular lymphocytic infiltrates (Fig. 4, left). Increases in actin RNA in the lungs from all acutely infected animals (Fig. 2, top) probably arise from these infiltrating inflammatory cells. However, it is important to note that the same difference in actin mRNA levels is seen in the lungs of control MLV/HIV-1[Δ NF- κ B] transgenic mice with no increase in the appearance of viral transcripts. RNA extracted from lungs of infected mice also contained elevated levels (5–34-fold increases after normalization with HPR T

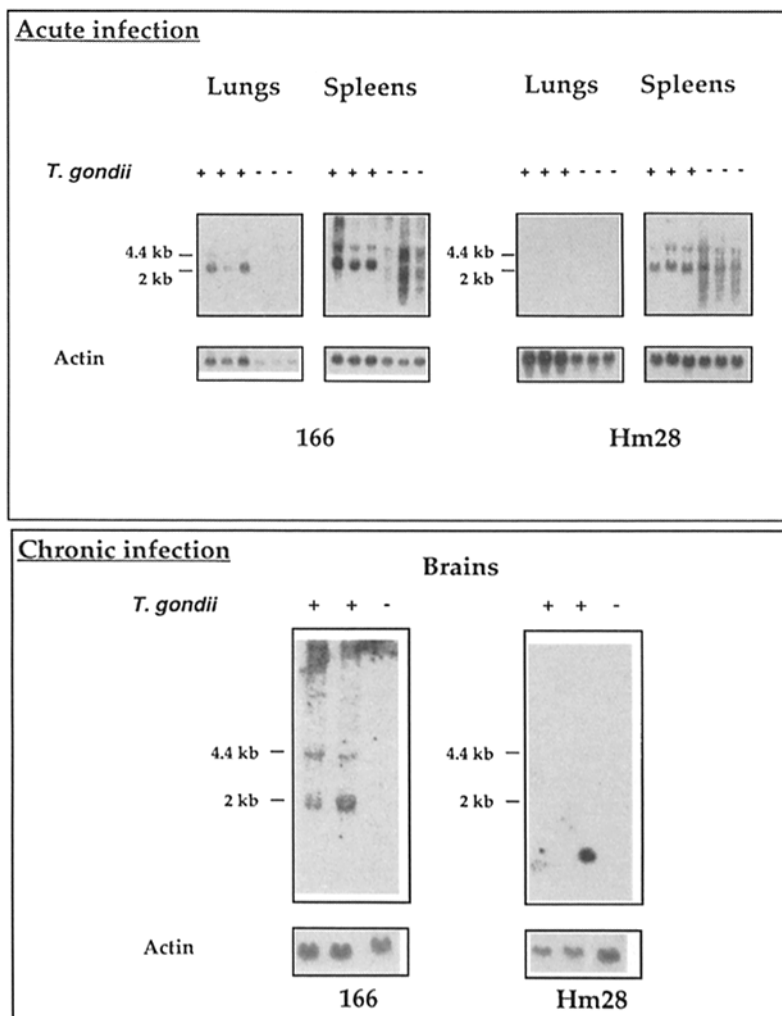


Figure 2. Transgenic mice were injected intraperitoneally with 20 *T. gondii* cysts (0.5 ml brain homogenate)/mouse or brain homogenate alone (mock-infected animals). Animals were killed after 10 (acute phase, top) or 50 d (chronic phase, bottom) and total RNA was prepared from different tissues. Acute infection of HIV-1 transgenic mice (line 166 homozygous mice) with *T. gondii* resulted in increased levels of HIV-1-specific transcripts in the lungs and spleens as detected by Northern blot analysis of total RNA (20 μg RNA/lane). In contrast, acute infection of MLV/HIV-1[Δ NF- κ B] (Hm28) transgenic mice resulted only in a slight increase in HIV-1 transcripts associated with spleen but not lung. Northern blot analysis of brain extracts of chronically infected HIV-1 and MLV/HIV-1 [Δ NF- κ B] transgenic mice demonstrated increased HIV-1 transcription in HIV-1 (166) but not MLV/HIV-1[Δ NF- κ B] (Hm28) transgenic mice. Each lane represents an individual animal. Hybridization with an actin probe is shown to provide an internal standard with a known housekeeping gene. A repeat experiment with six additional mice gave similar results.

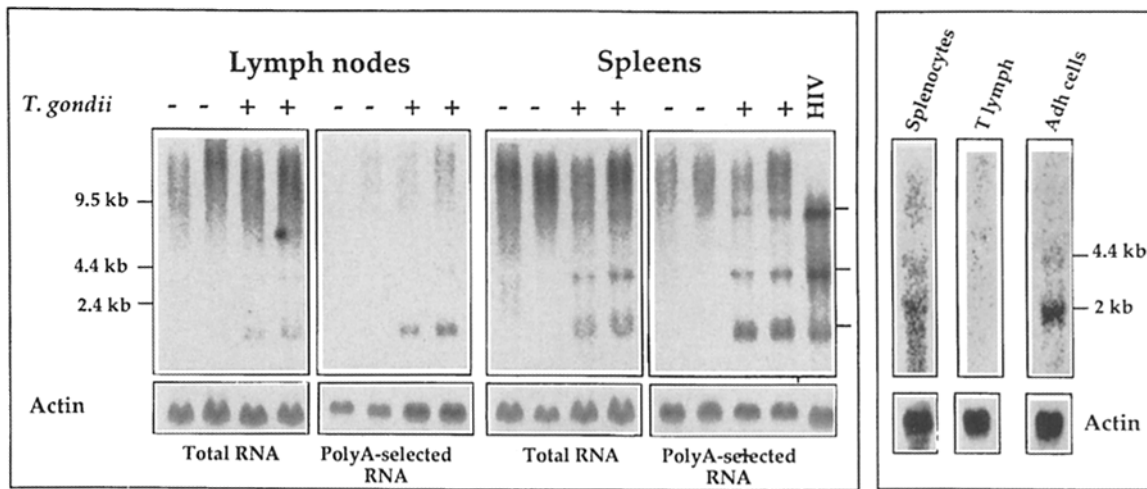


Figure 3. (Left) RNA was extracted from lymph nodes (cervical and axillary) or spleens of line 166 mice during the acute phase of *T. gondii* infection, and Northern analysis was performed under identical conditions before and after purification of mRNA on an oligo-dT column. Control HIV-1 RNA was prepared from human PBMC 3 d after infection with virus derived from pNL4-3 transfections of CD4⁺ HeLa cells. (Right) Total spleen cells were collected from 166 HIV-1 transgenic mice, 10 d after infection with *T. gondii*, and enriched for either T lymphocytes or macrophage-adherent cells. The expression of HIV-1 transcripts in unfractionated splenocytes, T cell-enriched fraction, and the adherent cell population was then compared by Northern blot analysis. The expression of CD3 marker in spleen cells was 30% and >90%, respectively, before and after T cell enrichment. The percentage of cells expressing the MAC-1 marker was 32% before and 82% after isolation of adherent population. The results shown are representative of three separate experiments performed.

mRNA) of transcripts for inflammation-associated monokines (IL-1, IL-6, TNF- α , and GM-CSF) and a tachyzoite-specific gene (SAG-2) (Fig. 4, right).

After the development of immunity, tachyzoites are cleared from host tissues and necrotic foci regenerate. The chronic phase of infection ensues and the parasite characteristically appears in the form of bradyzoites contained within cysts found predominantly in the central nervous system. Occasionally tachyzoites may escape from cysts and probably boost the immune response that effectively controls the further dissemination of the parasite (19, 37). Elevations in tachyzoite mRNA were detected in brains of infected control or transgenic mice in the chronic phase (30–50 d) of infection (Fig. 5, right). In contrast to the same tissues in acutely infected animals (data not shown), brains of chronically infected line 166 transgenic mice displayed marked increases in HIV-1-specific RNA (Fig. 2, bottom). By contrast, HIV-1 transcripts were not observed in brain extracts of line Hm28 (MLV/HIV-1[Δ NF- κ B]) transgenic mice chronically infected with *T. gondii*. Identical results were obtained with the second line (Hm54) of MLV/HIV-1[Δ NF- κ B] animals (data not shown). Chronic disease in the brain tissues of HIV-1 transgenic (as well as control) mice was characterized by intense inflammatory infiltrates and elevations (23–97-fold after normalization with HPRT mRNA) of monokine (IL-1, IL-6, TNF- α , and GM-CSF) mRNAs (Fig. 5). It is interesting to note that the grade of necrosis (1.3 ± 1.1) scored in brain sections of chronically infected 166 mice was significantly greater than that (0.2 ± 0.1) determined for infected nontransgenic FVB/N animals.

Characterization of HIV-1 RNA and Identification of Cellular Source of HIV-1 Transcripts. HIV-1-related RNA was de-

tected in spleens of uninfected HIV-1 transgenic mice, but it was smeared and of no discrete size (Fig. 2 and Fig. 3, left) (30). This constitutively expressed HIV-1 RNA in lymphoid tissue of HIV-1 transgenic mice had the characteristics of run-on transcripts. The high molecular weight HIV-1-related nucleic acid (Figs. 2 and 3) was RNA based on its sensitivity to RNase and on its insensitivity to DNase digestion (data not shown). After infection with *T. gondii*, discrete HIV-1 transcripts appeared in the spleen and lymph nodes but some high molecular weight RNA persisted (Figs. 2 and 3). After the selection of poly(A) RNA, typical HIV-1 transcripts (i.e., 2.0, 4.3, and 9.2 kb) were enriched in cell extracts. In addition, the results presented in Fig. 3 (left) show that the discrete HIV-1 transcripts from *T. gondii*-infected mice comigrated with HIV-1 transcripts present in productively infected PBMC.

Increases in tissue-associated HIV-1 RNA after *T. gondii* infection arose either from the activation of HIV-1 transcription in resident cells or from the infiltration of lung or brain by inflammatory cells expressing HIV-1 RNA. To investigate the cell source of HIV-1 transcription, we fractionated spleen cells from HIV-1 transgenic mice acutely infected with *T. gondii*. Splenocytes (36% T cells, 30% B cells, and 32% macrophages) harvested 10 d after infection were separated into T lymphocytes (>90% purity) and adherent cells (~82% macrophages) and analyzed by FACS[®] as previously described (31). As shown in Fig. 3 (right), HIV-1 transcription in total splenocytes was detected in the macrophage-enriched adherent cell population but not in the T cell-enriched population.

Induction of HIV-1 Transcripts by *T. gondii* In Vitro. The ability of macrophages from HIV-1 transgenic mice to express

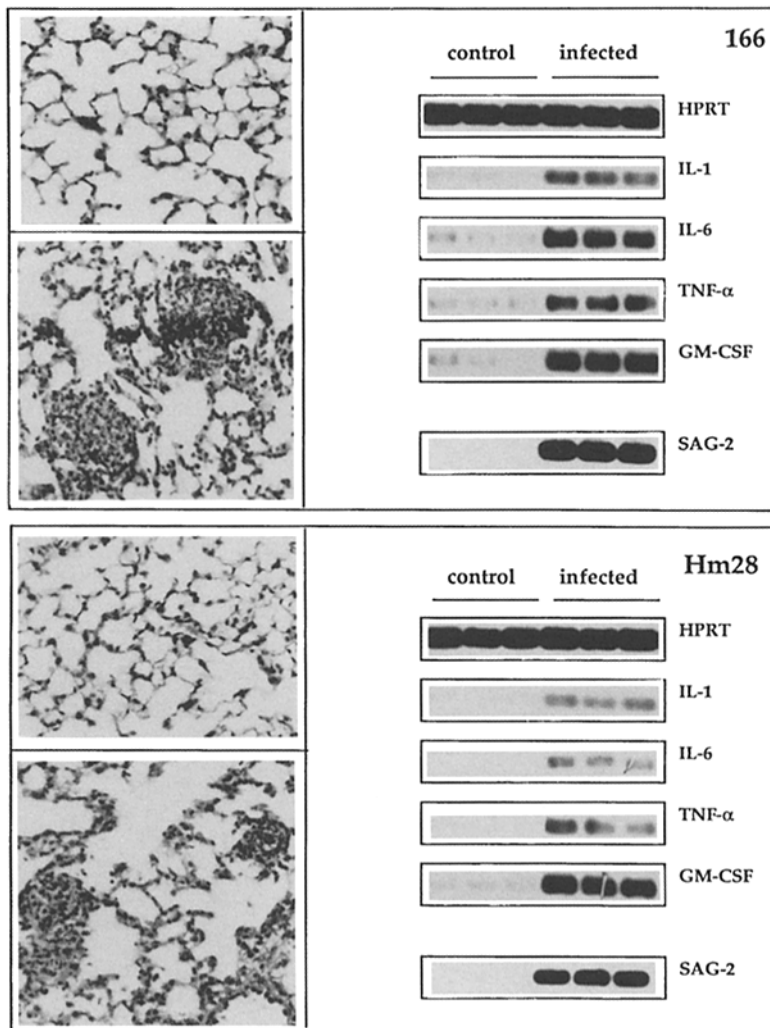


Figure 4. (Right) Detection by RT-PCR of mRNAs for the cytokines IL-1, IL-6, TNF- α , and GM-CSF, as well as a tachyzoite-specific gene (*SAG-2*) in lungs of HIV-1 (166) and MLV/HIV-1 [Δ NF- κ B] (*Hm28*) mice acutely infected with *T. gondii* (ME-49 strain). The blot shows Southern hybridization patterns of the PCR products with their respective internal oligonucleotide probes of RNA samples from lungs of individual control (noninfected animals) or mice infected for 10 d. HPRT is shown as a control housekeeping gene. Three different animals were assayed in each group. Similar results were obtained in a second experiment. (Left) H&E stained sections of lung tissues before (upper photomicrographs) and after infection (lower micrographs) showing characteristic inflammatory infiltrates that were of similar intensity in the two different transgenic mouse lines. $\times 320$.

HIV-1 RNA was investigated further in thioglycolate-elicited populations of peritoneal macrophages, activated *ex vivo* with known inducers of HIV-1 LTR-directed transcription. Significantly, whereas a soluble extract of tachyzoites (STAg) induced HIV-1 transcription *ex vivo* in macrophages from line 166 transgenic mice containing the wild-type HIV-1 LTR, it had no effect on elicited macrophages from line Hm28 (MLV/HIV-1[Δ NF- κ B]) mice (Fig. 6 A, left). As expected, LPS and TNF- α also induced expression of HIV-1-specific mRNAs in inflammatory macrophages (Fig. 6 A, right); STAg, under the conditions employed, equalled or surpassed these agents inducing as much as fivefold (after normalization with actin RNA levels) increases in HIV-1 RNAs. Whereas transcription of HIV-1 RNA was not induced by LPS, STAg, or TNF- α in inflammatory macrophages from the two independent MLV/HIV-1[Δ NF- κ B] transgenic lines (Hm28 and Hm54), expression was confirmed in two additional HIV-1 transgenic mouse lines that contain the wild-type LTR (designated Δ Gag/Pol [26, 27] and Δ Gag/Pol/Nef) (data not shown). Similar transcription results were obtained when bone marrow-derived macrophages obtained from the same MLV/

HIV-1[Δ NF- κ B] or HIV-1 transgenic mouse strains were stimulated with either STAg, LPS, or TNF- α (data not shown).

Role of TNF- α and NF- κ B in HIV-1 Expression Induced by T. gondii. To examine mechanisms by which *T. gondii* could activate the HIV-1 LTR in the inflammatory macrophages from HIV-1 transgenic mice, we assayed culture media for the increased production of TNF- α after the activation of macrophages. Transgenic macrophages were comparable to nontransgenic macrophages in their ability to produce and release TNF- α into the culture medium after STAg (Fig. 6 A, left) or LPS (data not shown) treatment. Comparable levels of TNF- α were produced by macrophages from either the wild-type HIV-1 or MLV/HIV-1[Δ NF- κ B] transgenic lines after activation with different stimuli. These results suggest that the inability of macrophages from MLV/HIV-1[Δ NF- κ B] transgenic mice to express HIV-1 transcripts is not due to a generalized defect in activation and/or TNF- α synthesis by these cells. TNF- α is known to activate the HIV-1 LTR (7–11) in a variety of different systems. Therefore, we cultured macrophages from the HIV-1 transgenic mice with different stimuli (i.e., STAg, LPS, or

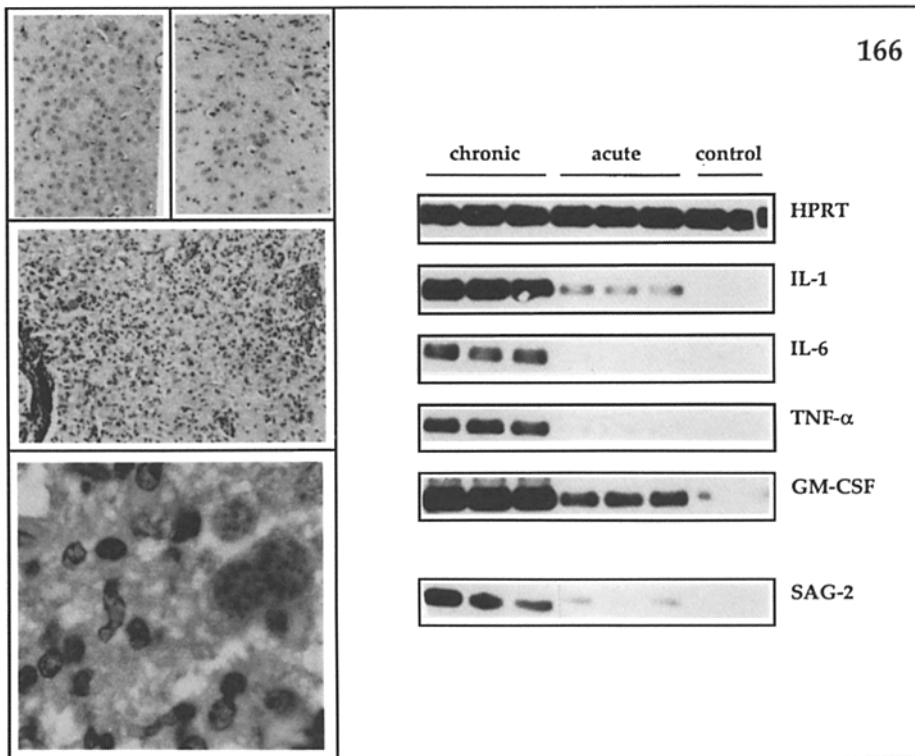


Figure 5. Detection of HPR1, IL-1, IL-6, TNF- α , GM-CSF, and SAG-2 mRNAs in brain extracts of HIV-1 transgenic (166) mice acutely (10-d) or chronically (50-d) infected with *T. gondii* using methods similar to those described in Fig. 4. Similar results were obtained in a second experiment. Brain sections (stained with H&E) of noninfected and infected transgenic (line 166) mice are shown on the left: (top left) brain section from uninfected mouse ($\times 320$); (top right) section from acutely infected (10-d) mouse ($\times 320$) showing mild inflammatory infiltrate; (middle) equivalent section from chronically infected (50-d) mouse displaying intense inflammation; (bottom) higher magnification ($\times 3,000$) micrograph of brain section from same animal showing three parasite cysts (upper right) and surrounding inflammatory infiltrate. The intensities of inflammatory infiltrates, expression of cytokine mRNAs, necrosis and cyst numbers were clearly augmented in mice chronically infected as compared with those acutely infected with *T. gondii*.

TNF- α) in the presence of anti-TNF mAb. In vitro neutralization of TNF blocked the activation of HIV-1 transcription by TNF- α but only partially blocked STAg- or LPS-induced activation (Fig. 6 A, right).

The ability of TNF- α to stimulate HIV-1 expression is attributed, at least in part, to the activation of NF- κ B binding activity (7). To further analyze this question, NF- κ B induction in control and activated macrophages from FVB/N and the two types of transgenic mice was measured in an EMSA (34) using double-stranded DNA oligonucleotide probes containing either a NF- κ B consensus binding sequence (PD- κ B; Fig. 6 C, right; 35) or an HIV-1-derived NF- κ B binding motif (HIV-1- κ B; 4; Fig. 6 B, left). Increased NF- κ B binding activity in macrophages was observed when cells were stimulated with either STAg, LPS, or TNF- α . The identification of NF- κ B binding activity was confirmed in mobility shift assays by demonstrating the sensitivity of the binding complex to supershifting using commercial antibodies (types C-20 or A) directed against the p65 component of NF- κ B (Fig. 6 B). Under the conditions used, only the type A antibody cross-reacted with the murine NF- κ B component. The nonreactive antibody (C-20) was included as a negative control for nonspecific Ig binding. Similar levels of LPS- or STAg-stimulated NF- κ B induction were observed in inflammatory macrophages derived from nontransgenic (Fig. 6, B and C) and HIV-1 transgenic mice (Fig. 6 C). Anti-TNF antibodies, while only partially blocking the activation of NF- κ B by STAg and LPS, completely inhibited NF- κ B induction by TNF- α (Fig. 6 B). Thus, the induction of NF- κ B and HIV-1 transcription in macrophages from HIV-1 transgenic mice ap-

pears to share the same sensitivity to anti-TNF- α inhibition (compare in Fig. 6, A to B).

Discussion

In an attempt to develop an animal model to study HIV-1 pathogenesis and the function of viral genes in vivo, several lines of transgenic mice have been constructed (25–27). These animals provide a model for the postintegration steps of the HIV-1 life cycle only. Any infectious virus progeny generated in transgenic mice harboring full-length copies of the viral genome would be unable to establish a spreading infection in the mouse for several reasons including: (a) the failure of HIV-1 to adsorb and enter lymphocytes bearing mouse CD4 (38); and (b) the relative inefficiency, in mouse cells, of the HIV-1-encoded Tat and Rev proteins to direct levels of steady state RNA and transport unspliced and singly-spliced viral RNAs from the nucleus to the cytoplasm, respectively (39–41). Expression directed by the wild-type LTR in mouse cells or previously described transgenic mice is therefore very low (25–27). Furthermore, the pattern of HIV-1 expression in mouse cells (39) or most tissues from transgenic mice, as monitored by Northern blot hybridization, is reminiscent of a “Rev minus” phenotype, i.e., predominantly multiply spliced 2.0-kb transcripts and little full-length 9.2- and 4.0-kb singly spliced mRNAs. Nevertheless, depending on the degree of proviral expression, tissue origin of the samples, and possibly the extent of viral mRNA enrichment, the profile observed in the Northern blot may vary greatly. As shown in Fig. 1, the three classes of HIV-1 RNA are clearly evident in samples puri-

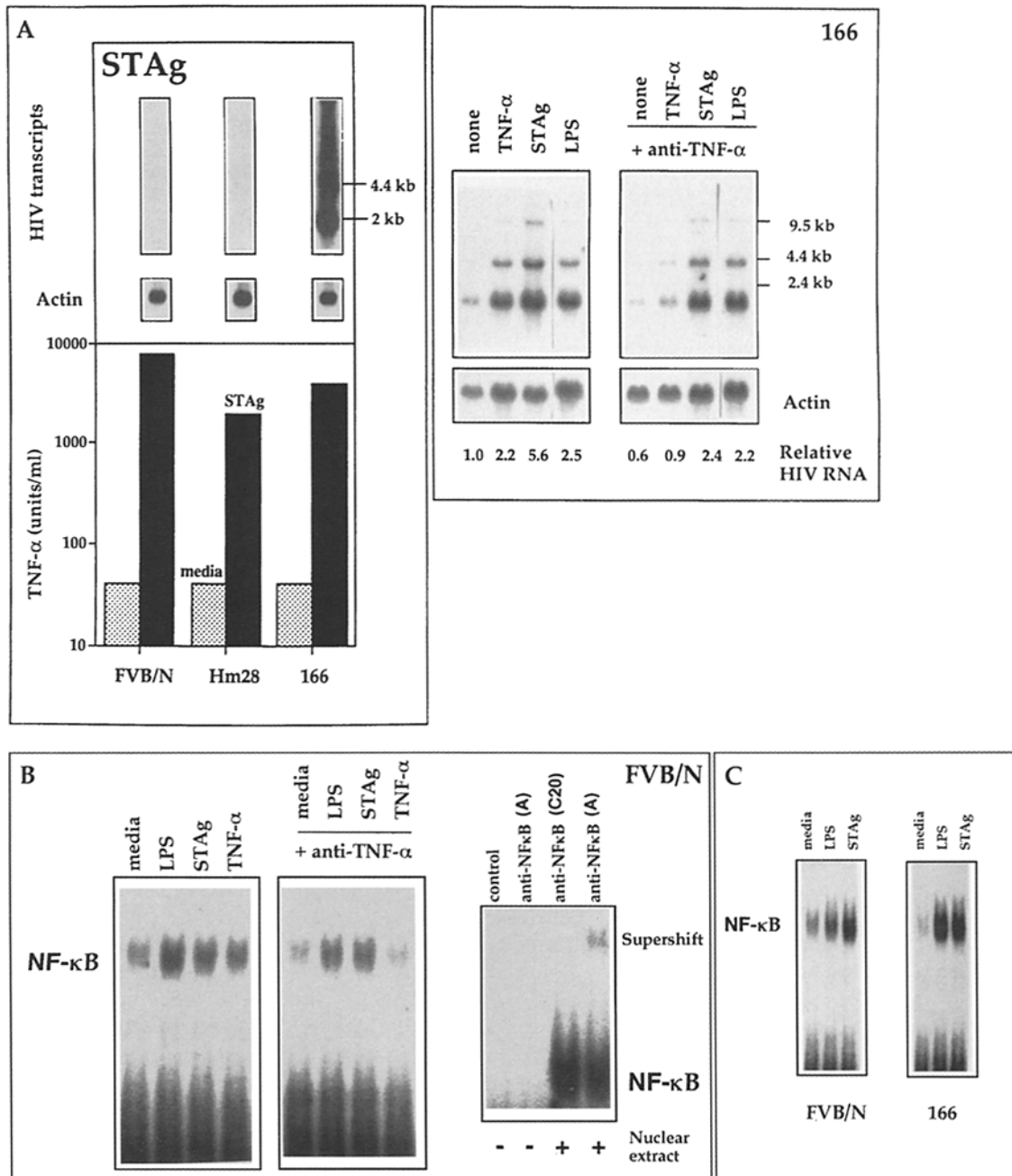


Figure 6. (A) (Left) HIV-1 RNA induced by *T. gondii* extracts in thioglycolate-elicited macrophages from HIV-1, MLV/HIV-1[Δ NF- κ B] transgenic and nontransgenic littermates. Peritoneal macrophages were treated with tachyzoite extract (STAg, 5 μ g/ml) and total RNA prepared 10 h later. The samples (5 μ g RNA/lane) were analyzed for HIV-1 or actin-specific RNAs by Northern analysis (top). TNF- α levels were measured in culture supernatants from the same macrophages stimulated with STAg or left in media (bottom) using the L939 bioassay. (Right) Thioglycolate-elicited macrophages were prepared as described above and exposed to either *E. coli* LPS (100 ng/ml, serotype 055:B5; Sigma Chemical Co.), *T. gondii* extract (5 μ g/ml) or TNF- α (100 U/ml), in the presence of either control (GL-113 anti- β -galactosidase; 20 μ g/ml) or anti-TNF- α (20 μ g/ml) mAbs as indicated. HIV-1 transcripts were measured by Northern analysis and the intensities of the hybridization bands compared by densitometry and normalized with the signals given by actin RNA. (B) Nuclear extracts of peritoneal macrophages from FVB/N mice stimulated in vitro with *T. gondii* extract as above were assayed for the presence of NF- κ B binding activity in EMSAs as described in Materials and Methods. The HIV-1- κ B (4) double-stranded oligonucleotide probe was used to detect the NF- κ B binding activity. Supershift antibodies (types C-20 or A) were obtained from Santa Cruz Biotechnology, Inc. and used according to the manufacturer's protocols. The reaction of the probe is shown in the presence (+) or absence (-) of the nuclear extract. (C) Comparison of LPS- or STAg-induced NF- κ B activity in inflammatory macrophages derived from nontransgenic (FVB/N) versus HIV-1 transgenic (166) mice using the double-stranded oligonucleotide probe, PD- κ B (35). The experimental conditions were similar to those described in B.

fied from the lens of the two transgenic mouse lines under study in this report. In contrast, expression directed by the wild-type LTR in most other mouse tissues is extremely low and the RNA species observed are frequently limited to the fully spliced 2-kb class (Fig. 2).

In the present study, we have shown that in vivo infection with the opportunistic pathogen, *T. gondii*, leads to induction of HIV-1 mRNAs in tissues in which constitutive proviral expression is normally at the limit of detectability. The exposure of macrophages to parasite extracts in vitro enhances the level of HIV-1 RNAs, arguing that these cells are likely to be a major target for parasite-induced proviral activation. It is interesting to note that, in addition to multiply spliced low molecular weight transcripts, singly spliced 4.2- and unspliced 9.0-kb viral mRNAs are evident in some *T. gondii*-exposed tissues, suggestive evidence of partial Rev function.

We postulate that infection of HIV-1 transgenic mice with *T. gondii* potentiates the expression of HIV-1 mRNA in inflammatory cells as shown ex vivo in macrophages. During *Toxoplasma* infection, infiltration of tissues (e.g., lungs and brain that normally display no or negligible levels of HIV-1 proviral expression) by inflammatory cells results in the synthesis of measurable levels of HIV-1-specific RNAs. These HIV-1 transcripts are likely to represent products of the cells migrating to the site of *T. gondii* infection rather than those preexisting in the tissue. The latter concept also suggests that a pathogen-induced inflammatory response may play an important role in increasing HIV-1 levels in specific tissue sites. Similar phenomena may occur during infection with other opportunistic pathogens, such as *Mycobacterium tuberculosis* (42–44) or *M. avium* (45) which also possess the ability to attract and activate macrophages into the infection site and therefore would promote HIV-1 expression and viral spread in vivo.

The data presented here also suggest that NF- κ B induction plays a central role in the activation of HIV-1 transcription in macrophages from HIV-1 transgenic mice by *T. gondii*. The failure to activate HIV-1 transcription in macrophages from two independent MLV/HIV-1[Δ NF- κ B] transgenic mouse lines lacking NF- κ B binding sites is consistent with the requirement of NF- κ B activity for in vivo activation of the HIV-1 LTR during infection with *T. gondii*. Furthermore, ex vivo activation experiments performed in the presence of anti-TNF antibodies suggest that

both TNF- α -dependent (7–11) and -independent mechanisms are involved in the HIV-1 LTR activation by *T. gondii* since neutralization of that cytokine caused only a minor inhibition in STAg-induced proviral transcription. The existence of TNF-independent mechanisms was also suggested by previous studies (46) indicating that direct exposure of a chronically infected monocytoid cell line to LPS or PMA results in an augmentation of HIV-1 replication in the absence of TNF- α .

Since toxoplasmosis can be associated with HIV-1 infection (17, 18), the results presented here imply that activation of macrophages by tachyzoites (during acute infection and/or reactivation of chronic disease) may augment viral gene expression and virus replication and lead to increased virus loads in HIV-1-seropositive individuals. However, to the best of our knowledge there is only one study suggesting that *T. gondii* may act as an HIV-1 cofactor. In the latter report, increased congenital transmission of HIV-1 was observed in children of *T. gondii*-infected mothers (47). Studies specifically evaluating HIV-1 progression in individuals infected with the protozoan are necessary to validate this hypothetical disease interaction.

A major limitation in studying the influence of coinfections on the activation of HIV-1 in vivo, and therefore on AIDS pathogenesis, is the absence of a suitable animal model. The results presented here suggest that transgenic mice containing the complete wild-type HIV-1 proviral DNA may serve as useful tools for investigating these phenomena as well as for testing effective therapies for the prevention or repression of HIV-1 transcription in vivo. Two important attributes of these transgenic mice are the inducibility of HIV-1 gene expression in macrophages, and that the initiation and/or augmentation of viral transcription in macrophages appears to be under the control of the wild-type HIV-1 LTR. In humans, macrophages represent a major reservoir for HIV-1. Thus, the activation of HIV-1-infected macrophages resulting in transcription of HIV-1 genes and viral replication could be an important element affecting disease progression (48–51). The HIV-1 transgenic mouse model described here should provide a useful tool for studying pathways responsible for HIV-1 induction in macrophages in vivo as well as for evaluating treatments that inhibit this potentially important mechanism of AIDS pathogenesis.

The authors gratefully acknowledge Cherise Fenton for her excellent technical assistance. We also thank Dr. Ulrich Siebenlist from the Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health for providing the oligonucleotide probes used in the NF- κ B binding assays and for his helpful discussions.

Address correspondence to Dr. Alan Sher, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 4, Room 126, 9000 Rockville Pike, Bethesda, MD 20892.

Received for publication 25 October 1995 and in revised form 2 February 1996.

References

- Embretson, J., M. Zupancic, J.L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A.T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature (Lond.)*. 362: 359–362.
- Pantaleo, G., C. Graziosi, J.F. Demarest, L. Butini, M. Montroni, C.H. Fox, J.M. Orenstein, D.P. Kotler, and A.S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature (Lond.)*. 362:355–358.
- Fauci, A.S. 1993. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science (Wash. DC)*. 262:1011–1018.
- Nabel, G., and D. Baltimore. 1988. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (Lond.)*. 326:711–713.
- Garcia, J.A, F.K. Wu, R. Mitsuyasu, and R.B. Gaynor. 1987. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3761–3770.
- Koyanagi, Y., W.A. O'Brian, J.Q. Zhao, D.W. Golde, J.C. Gasson, and I.S.Y. Chen. 1988. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. *Science (Wash. DC)*. 241:1673–1675.
- Osborn, L., S. Kunkel, and G.J. Nabel. 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of nuclear factor κ B. *Proc. Natl. Acad. Sci. USA*. 86:2336–2340.
- Folks, T.M., K. Clouse, J. Justement, A. Robson, E. Duh, J.H. Kerl, and A.S. Fauci. 1989. Tumor necrosis factor- α induces expression of human immunodeficiency virus in chronically infected T cell clone. *Proc. Natl. Acad. Sci. USA*. 86: 2365–2368.
- Matsuyama, T., Y. Hamamoto, G.I. Soma, D.I. Mizuno, N. Yamamoto, and N. Kobayashi. 1989. Cytocidal effect of tumor necrosis factor on cells chronically infected with human immunodeficiency virus (HIV-1): enhancement of HIV-1 replication. *J. Virol.* 63:2504–2509.
- Poli, G., A. Kinter, J.S. Justement, J.H. Kehrl, P. Bressler, S. Stanley, and A.S. Fauci. 1990. Tumor necrosis factor- α functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proc. Natl. Acad. Sci. USA*. 87:782–785.
- Poli, G., P. Bressler, A. Kinter, E. Duh, W.C. Timmer, A. Rabson, J.S. Justement, S. Stanley, and A.S. Fauci. 1990. Interleukin-6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor- α by transcriptional and post transcriptional mechanisms. *J. Exp. Med.* 172:151–158.
- Biswas, P., G. Poli, A.L. Kinter, J.S. Justement, S.K. Stanley, W.J. Maury, P. Bressler, J.M. Orenstein, and A.S. Fauci. 1992. Interferon γ induces the expression of human immunodeficiency virus in persistently infected promonocytic cells (U1) and redirects the production of virions to intracytoplasmic vacuoles in phorbol myristate acetate-differentiated U1 cells. *J. Exp. Med.* 176:739–750.
- Laurence, J. 1992. Viral cofactors in the pathogenesis of HIV disease. In *AIDS and other Manifestations of HIV Infection*. G.P. Wormser, editor. Raven Press, New York. 77–83.
- Montagnier, L. 1993. Mycoplasma as cofactor in AIDS. In *New Concepts in AIDS Pathogenesis*. L. Montagnier and M. L. Gougeon, editors. Marcel Dekker, Inc., New York. 115–125.
- Toossi, Z., J.G. Sierra-Madero, R.A. Blinkhorn, M.A. Mettler, and E.A. Rich. 1993. Enhanced susceptibility of blood monocytes from patients with pulmonary tuberculosis to productive infection with human immunodeficiency virus type 1. *J. Exp. Med.* 177:1511–1516.
- Brown, C.C., G. Poli, N. Lubaki, M. St. Louis, F. Davachi, L. Musey, T. Manzila, A. Kovacs, T.C. Quinn, and A.S. Fauci. 1994. Elevated levels of tumor necrosis factor- α in Zairian neonate plasmas: implications for perinatal infection with the human immunodeficiency virus. *J. Infect. Dis.* 169: 975–980.
- Luft, B.J., R.G. Brooks, F.K. Conley, R.E. McCabe, and J.S. Remington. 1984. Toxoplasmic encephalitis in patients with acquired immune response deficiency syndrome. *JAMA (J. Am. Med. Assoc.)*. 252:913–917.
- Navia, B.A., C.K. Petito, J.W.M. Gold, E.S. Cho, B.D. Jordan, and R.W. Price. 1986. Cerebral toxoplasmosis complicating the acquired immune deficiency syndrome: clinical and neuropathologic findings in 27 patients. *Ann. Neurol.* 19:224–238.
- Gazzinelli, R.T., E.Y. Denkers, and A. Sher. 1993. Host resistance to *Toxoplasma gondii*: a model for studying the selective induction of cell-mediated immunity by intracellular parasites. *Infect. Agents Dis.* 2:139–149.
- Gazzinelli, R.T., S. Bala, R. Stevens, M. Baseler, L. Wahl, J. Kovacs, and A. Sher. 1995. HIV infection suppresses type-1 lymphokine and IL-12 responses to *Toxoplasma gondii* but fails to inhibit synthesis of other parasite induced monokines. *J. Immunol.* 155:1565–1574.
- Gazzinelli, R.T., S. Hieny, T. Wynn, S. Wolf, and A. Sher. 1993. IL-12 is required for the T-cell independent induction of IFN- γ by an intracellular parasite and induces resistance in T-deficient hosts. *Proc. Natl. Acad. Sci. USA*. 90:6115–6119.
- Gazzinelli, R.T., M. Wysocka, S. Hayashi, E.Y. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher. 1994. Parasite-induced IL-12 stimulates early IFN- γ synthesis and resistance during acute infection with *Toxoplasma gondii*. *J. Immunol.* 153:2533–2543.
- Li, Z.Y., C.L. Manthey, P.Y. Perera, A. Sher, and S.N. Vogel. 1994. *Toxoplasma gondii* soluble antigen induces a subset of lipopolysaccharide-inducible genes and tyrosine phosphoproteins in peritoneal macrophages. *Infect. Immun.* 62:3434–3440.
- Bala, S., G. Englund, J. Kovacs, L. Wahl, M. Martin, A. Sher, and R.T. Gazzinelli. 1994. *Toxoplasma gondii* soluble products induce cytokine secretion by macrophages and potentiate in vitro replication of a monotropic strain of HIV. *J. Eukaryot. Microbiol.* 41:7S.
- Leonard, J.M., J.W. Abramczuk, D.S. Pezen, R. Rutledge, J.H. Belcher, F.T. Hakim, G.M. Shearer, L. Lamperth, W. Travis, T. Fredrickson et al. 1988. Development of disease and virus recovery in transgenic mice containing HIV proviral DNA. *Science (Wash. DC)*. 242:1665–1670.
- Dickie, P., F. Ransdell, A.L. Notkins, and S. Venkatesan. 1993. Spontaneous and inducible epidermal hyperplasia in transgenic mice expressing HIV-1 Nef. *Virology*. 197:431–438.
- Dickie, P., J. Felser, M. Eckhaus, J. Bryant, J. Silver, N. Marinis, and A.L. Notkins. 1991. HIV associated nephropathy in transgenic mice expressing HIV-1 genes. *Virology*. 185:109–119.
- Taketo, M., A.C. Schroeder, L.E. Mobraaten, K.B. Gunning, G. Hanten, R.R. Fox, T.H. Roderick, K.B. Stewart, F. Lilly, C.T. Hansen, and P.A. Overbeek. 1991. FVB/N: an

- inbred mouse strain preferable for transgenic analyses. *Proc. Natl. Acad. Sci. USA*. 88:2065–2069.
29. Chang, L.J., E. McNulty, and M.A. Martin. 1993. Human immunodeficiency viruses containing heterologous enhancer/promoters are replication competent and exhibit different lymphocyte tropisms. *J. Virol.* 67:743–752.
 30. Muesing, M.A., D.H. Smith, and D.J. Capon. 1987. Regulation of mRNA accumulation by a human immunodeficiency virus trans-activator protein. *Cell.* 48:691–701.
 31. Gazzinelli, R.T., Y. Xu, S. Hieny, A. Cheever, and A. Sher. 1992. Simultaneous depletion of CD4⁺ and CD8⁺ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J. Immunol.* 149:175–180.
 32. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
 33. Sakaguchi, M., B. Zenzie-Gregory, J.E. Groopman, S.T. Smale, and S. Kim. 1991. Alternative pathway for induction of human immunodeficiency virus gene expression: involvement of the general transcription machinery. *J. Virol.* 65:5448–5456.
 34. Bours, V., P. Burd, K. Brown, J. Villalobos, S. Park, R.P. Ryseck, R. Bravo, K. Kelly, and U. Siebenlist. 1992. A novel mitogen-inducible gene product related to p50/p105-NF- κ B participates in transactivation through a κ B site. *Mol. Cell. Biol.* 12:685–695.
 35. Ballard, D.W., W.H. Walker, S. Doerre, P. Sista, J.A. Moliter, E.P. Dixon, N.J. Pfeffer, M. Hamink, and W.C. Greene. 1990. The V-rel oncogene encodes a κ B enhancer binding protein that inhibits NK- κ B function. *Cell.* 63:803–814.
 36. Gazzinelli, R.T., I.A. Eltoun, T. Wynn, and A. Sher. 1993. Acute cerebral toxoplasmosis is induced by in vivo neutralization of TNF- α and correlates with down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. *J. Immunol.* 151:3672–3681.
 37. Frenkel, J.K. 1988. Pathophysiology of toxoplasmosis. *Parasitol. Today.* 4:273–278.
 38. Maddon, P.J., A.G. Dalgleish, J.S. McDougal, P.R. Clapham, R.A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell.* 47:333–348.
 39. Winslow, B.J., and D. Trono. 1993. The blocks to human immunodeficiency virus type 1 Tat and Rev functions in mouse cell lines are independent. *J. Virol.* 67:2349–2354.
 40. Hart, C.E., C. Ou, J.C. Galphin, L.T. Moore, L.T. Bachler, J.J. Wasmuth, S.R. Petteway, and G. Schochetman. 1989. Human chromosome 12 is required for HIV-1 expression in human-hamster hybrid cells. *Science (Wash. DC)*. 246:488–491.
 41. Newstein, M., E.J. Stanbridge, G. Casey, and P.R. Shank. 1990. Human chromosome 12 encodes a species-specific factor, which increases human immunodeficiency virus type I Tat-mediated trans-activation in rodent cells. *J. Virol.* 64:4565–4567.
 42. Chan, J., X. Fan, S.W. Hunter, P.J. Brennan, and B.R. Bloom. 1991. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. *Infect. Immun.* 59:1755–1761.
 43. Takashima, T., C. Ueta, I. Tsuyugushi, and S. Kishimoto. 1990. Production of tumor necrosis factor- α by monocytes from patients with pulmonary tuberculosis. *Infect. Immun.* 58:3286–3292.
 44. Roach, T.I., C.H. Barton, D. Chatterje, and J.M. Blackwell. 1993. Macrophage activation: lipoarabinomannan from avirulent and virulent strains of *Mycobacterium tuberculosis* differentially induces early genes cFos, KC, JE and tumor necrosis factor- α . *J. Immunol.* 150:1886–1896.
 45. Newman, G.W., T.G. Kelley, H. Gan, O. Kandil, M.J. Newman, P. Pinkston, R.M. Rose, and H. Remold. 1993. Concurrent infection of human macrophages with HIV and *Mycobacterium avium* results in decreased cell viability, increased *M. avium* multiplication and altered cytokine production. *J. Immunol.* 151:2261–2272.
 46. Pomerantz, R.J., M.B. Feinberg, D. Trono, and D. Baltimore. 1990. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. *J. Exp. Med.* 172:253–261.
 47. Kovacs, A.A.S., F. Hofman, S. Walker, J. Rhupp, S. Wong, D. Pavlova, D. Hinton, and S. Rasheed. 1993. Clinical and virologic predictors of maternal-infant transmission of HIV-1. *Abstr. Ann. Meet. Infect. Dis. Soc. Am.* 31a. (Abstr.)
 48. Koenig, S., H.E. Gendelman, J.M. Orenstein, M.C. Dal Canto, G.M. Pezeshkpour, M. Yungbluth, F. Janotta, A. Akshmit, M.A. Martin, and A.S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science (Wash. DC)*. 233:1089–1093.
 49. Salahuddin, S.Z., R.M. Rose, J.E. Groopman, P.D. Markham, and R.C. Gallo. 1986. Human T lymphotropic virus type III infection of human alveolar macrophages. *Blood.* 68:281–284.
 50. Gartner, S., P. Markovits, D.M. Markovits, M.H. Kaplan, R.C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science (Wash. DC)* 233:215–219.
 51. McElrath, M.J., J.E. Pruetz, and Z.A. Cohn. 1989. Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections. *Proc. Natl. Acad. Sci. USA.* 86:675–679.